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Cystotheca kusanoi comb. nov.: a redescription with new morphological observations

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Abstract—Conspecific Korean and Japanese powdery mildew specimens (including the type of *Sphaerotheca kusanoi*) on *Quercus* spp. previously identified as *Cystotheca lanestris* have been revealed as morphologically and phylogenetically distinct from North American *C. lanestris* specimens. The Asian specimens are confirmed as a separate species under the new combination *Cystotheca kusanoi*. *Cystotheca kusanoi* lacks distinct fibrosin bodies, even in fresh conidia and conidiophores. A key to *Cystotheca* spp. is provided.

Key words—*Cystotheceae, Erysiphaceae, Erysiphales,* oaks

Introduction

Cystotheca (*Erysiphaceae*, tribe *Cystotheceae*) is known to infect 62 host species, all except one belonging to *Fagaceae*. The genus is distinguished from other genera by the presence of special aerial hyphae (although these are absent in *C. esetacea* Z.X. Chen & Y.J. Yao) and the presence of two distinct layers in the chasmothecial wall (Braun & Cook 2012). In addition, conidia and conidiophores of *Cystotheca* species have been described as containing well-developed fibrosin bodies (Homma 1937, Braun 1987, Nomura 1997, Braun & Cook 2012).

Eight *Cystotheca* species have been recorded worldwide: *C. esetacea*, *C. indica* M.S. Patil & Maham., *C. lanestris* (Harkn.) Miyabe, *C. nanyuensis* (J.L. Zhou) U. Braun, *C. quercina* N. Ahmad & al., *C. tjibodensis* (Gäum.) Katum., *C. wrightii* Berk. & M.A. Curtis (Braun & Cook 2012), and *C. castanopsidis* (Meeboon & S. Takam.) Meeboon & S. Takam. (Wijayawardene & al. 2017). All species are native in Asia except for *C. lanestris*, originally described from North America but later recorded also from Asia.

Cystotheca lanestris is prevalent on various hosts that belong to the *Fagaceae* in North America and Asia (Braun & Cook 2012, Farr & Rossman 2018). Since 1940 in Korea, *C. lanestris* has been found on several *Quercus* species (Anonymous 1940; Amano 1986; Shin 1991, 2000). However, an investigation of Korean specimens revealed that the fungus occurring on *Quercus* species in Korea does not correspond with *C. lanestris* previously described from North America (Shin 1991). Further study using fresh samples collected throughout Korea led to the conclusion that the Korean specimens differ morphologically from all previously described species of *Cystotheca*. In addition, Japanese collections of *C. lanestris* s. lat. on *Quercus* spp. are morphologically similar to Korean collections. Here we report molecular sequence analyses of Korean and Japanese isolates from *Quercus* spp., to determine whether their phylogenetic placement agrees with their morphological similarity.

Materials & methods

Sample collection

Surveys were conducted at random at all sites in Korea, depending on accessibility at the time of the visits. Sites where diseases were observed were visited multiple times between 2011 and 2016 to obtain sufficient material for analyses. All collected plant material was processed on the day and place of collection. Representative specimens were deposited in the Herbarium of the Korea University, Seoul, Korea (KUS), and previously deposited *Cystotheca* species were re-examined. Japanese herbarium specimens labelled as "*Cystotheca lanestris*" were provided by the Tokyo University of Agriculture, Tokyo, Japan (TUAT; 11 specimens) and the National Museum of Nature and Science, Tsukuba, Japan (TNS; 24 specimens, including the isotype of *Sphaerotheca kusanoi*).

Morphological observation

The fungal fructification was detached from the infected leaves and mounted in a few drops of distilled water on a glass slide for light microscopy. Fungal structures were morphologically examined by bright field and differential interference contrast (DIC) light microscopy using an Olympus model BX51 microscope for measurements

and a Zeiss AX10 microscope equipped with a Zeiss AxioCam MRc5 for photography. The measurements were made at 100×, 200×, 400×, and 1000× magnifications.

DNA extraction, PCR amplification, and sequencing protocols

Genomic DNA was extracted from mycelia and chasmothecia using the Chelex 100 as previously described (Walsh & al. 1991, Hirata & Takamatsu 1996). The internal transcribed spacer (ITS) regions and large subunit (28S) ribosomal DNA were selected for the full survey. The ITS1+5.8S+ITS2 region was amplified using primers PMITS1 (5′-TCG-GAC-TGG-CCC-AGG-GAG-A-3′) and PMITS2 (5′-TCA-CTC-GCC-GTT-ACT-GAG-GT-3′) (Cunnington & al. 2003), while domains D1 and D2 of 28S rDNA were amplified using primers PM3 (5′-GKG-CTY-TMC-GCG-TAG-T-3′, Takamatsu & Kano 2001) and TW14 (5′-GCT-ATC-CTG-AGG-GAA-ACT-TC-3′, Mori & al. 2000).

The genomic DNAs served as a template for polymerase chain reaction (PCR). The PCR mixture contained 12.5 µl of 2x PCR buffer for KOD FX Neo, 5 µl of 2 mM of each dNTP, 0.375 µl of 20 mM of each primer, 0.5 µl of KOD FX Neo polymerase, and 1 µl of template DNA. The PCR products were checked on 1.2% agarose gels and examined under ultra-violet light. Sizes of the PCR products were estimated by comparison with a TaKaRa Bio 1 kb molecular weight standard DNA ladder. The PCR products were then purified using the Qiagen QIAquick PCR Purification Kit. The products were bidirectionally sequenced by a commercial sequencing company (Macrogen, Seoul, Korea) with the same primers used for PCR.

Sequence alignment and phylogenetic analyses

All obtained sequences were edited using DNASTAR Lasergene SeqMan and Editseq software version 7.1.0 and MEGA version 7 (Kumar & al. 2016). Sequences obtained in this study were deposited in GenBank. Alignments were made with an online version of MAFFT7 (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013). Phylogenetic trees were inferred from the maximum likelihood (ML) and maximum-parsimony (MP) methods in PAUP* 4.0, and Bayesian (BI) analyses in MRBAYES 3.1.2. ML analyses were performed using RAxML HPC BlackBox ver. 8.1.11 (Stamatakis 2014) using the default option with the generalized time reversible (GTR) substitution model implemented in the CIPRES cluster server (https://www.phylo.org/) at the San Diego Supercomputing Center. MP (Fitch 1971) analysis was carried out using PAUP* 4.0b10 (Swofford 2003). MP genealogies for a single gene were constructed using the heuristic search option with 100 random taxon additions and tree bisection and reconnection (TBR). Gaps were treated as missing data and all characters were unordered and of equal weight. Statistical support for nodes was obtained by performing 1000 bootstrap replicates. In addition, the consistency index (CI), retention index (RI), and tree length (TL) were determined for the resulting trees. ML inference was conducted using RAxML version 7.2.6 (Stamatakis 2014). The statistical selection of an evolutionary best-fit model of nucleotide substitution was carried out using jModelTest 0.1.1 and the Akaike's Information Criterion (AIC) (Darriba & al. 2012). The Bayesian inference (BI) analysis was performed for each individual data set based on a Markov chain Monte Carlo (MCMC) algorithm using

MrBayes version 3.2 (Ronquist & al. 2012). Two independent runs were performed simultaneously for 3 million generations every 100 generations. Burn-in values were determined with Tracer version 1.4 and the first 25% of the sampled trees representing the burn-in were discarded. Phylogenetic trees obtained from the BI and ML analyses were viewed in MEGA version 7 (Kumar & al. 2016).

Results

Morphology

The Korean and Japanese *Cystotheca* specimens on *Quercus* spp., including the isotype of *Sphaerotheca kusanoi*, were all morphologically similar but differed from *C. lanestris* from North America. We therefore propose a new combination for the Asian species:

Cystotheca kusanoi (Henn. & Shirai) S.E. Cho & H.D. Shin, **comb. nov.** Figs 1, 2

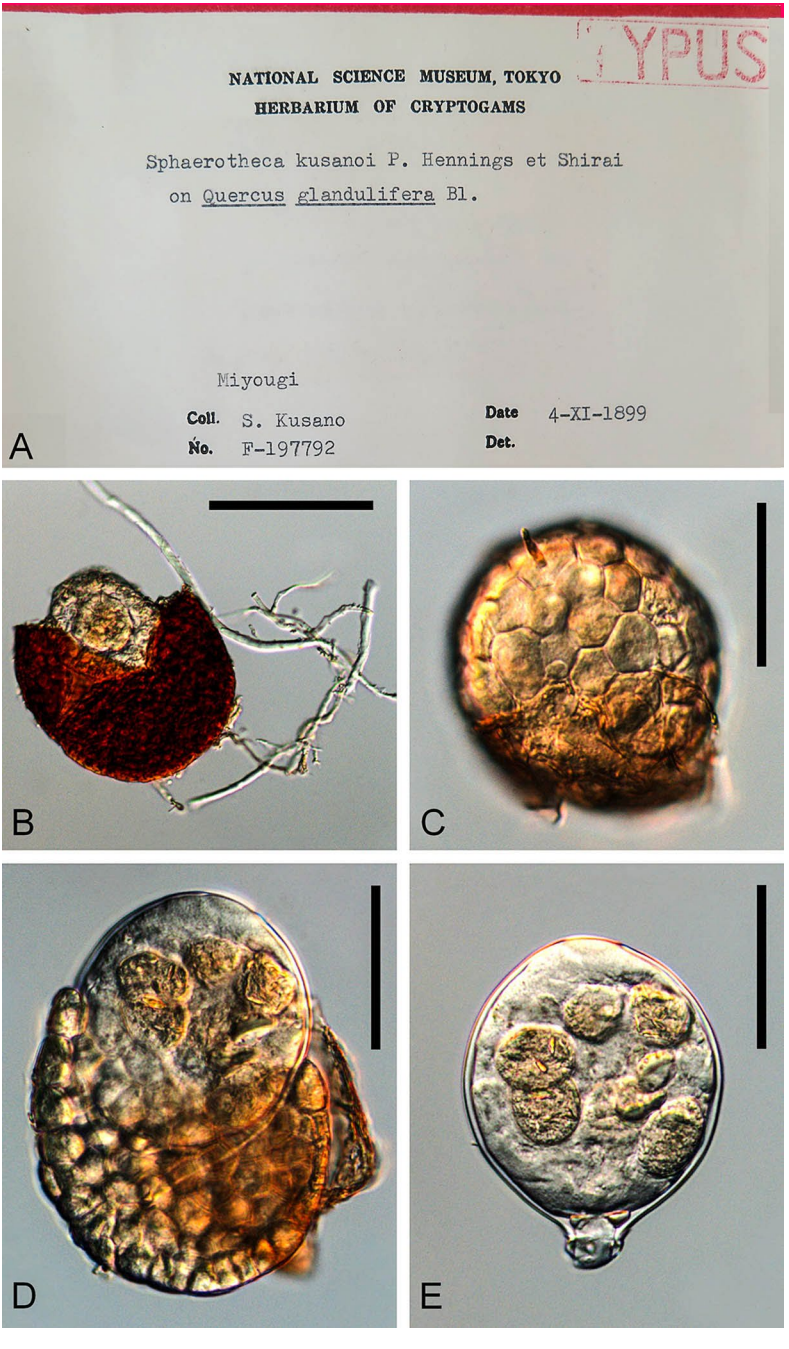
MycoBank MB 820131

≡ *Sphaerotheca kusanoi* Henn. & Shirai, Bot. Jahrb. Syst. 29(1): 147, 1900 ["1901"]. type: Japan, Prov. Kōzuke [Gunma Prefecture], Mt. Myogi, on living leaves of *Quercus glandulifera* Blume [= *Q. serrata* Murray], 4 Nov. 1899, S. Kusano no. 123 (TNS-F-197792, isotype).

"*Cystotheca tenuis*" Miyabe & Takah., in Ideta, Pract. Phytopathol.: 170, 1901, nom. nud.

Colonies hypophyllous, circular to irregular white patches, covering the whole lower leaf surface, at first whitish, later turning grayish to brown. Special aerial hyphae filiform, hyaline to pigmented, aseptate, 100–400 × 3.5–5 µm. Appressoria indistinct or maybe absent. Conidiophores arising from the upper part of mother cells, 110–175 × 10–12.5 µm, producing 2–4 immature conidia in chains with a sinuate outline. Foot-cells mostly straight or almost so, basal septum elevated 6–10 µm above junction with mother cells, 42.5–70 µm. Primary conidia conically rounded at the apex, subtruncate at the base. Secondary conidia doliiform, limoniform, hyaline, 27–38 × 17–23 µm, with a length/width ratio of 1.4–1.8, without conspicuous fibrosin bodies. Germ tubes very long, filiform, 0–1-septate, lateral to perihilar. Chasmothecia hypophyllous, scattered or gregarious, partly embedded in special aerial hyphae, dark brown, spherical, 70–95 µm diameter, containing a single ascus, rarely two asci per chasmothecium. Peridium consisting of two layers, outer wall composed of dark brown, polygonal cells, 10–20 µm wide. Inner wall composed of

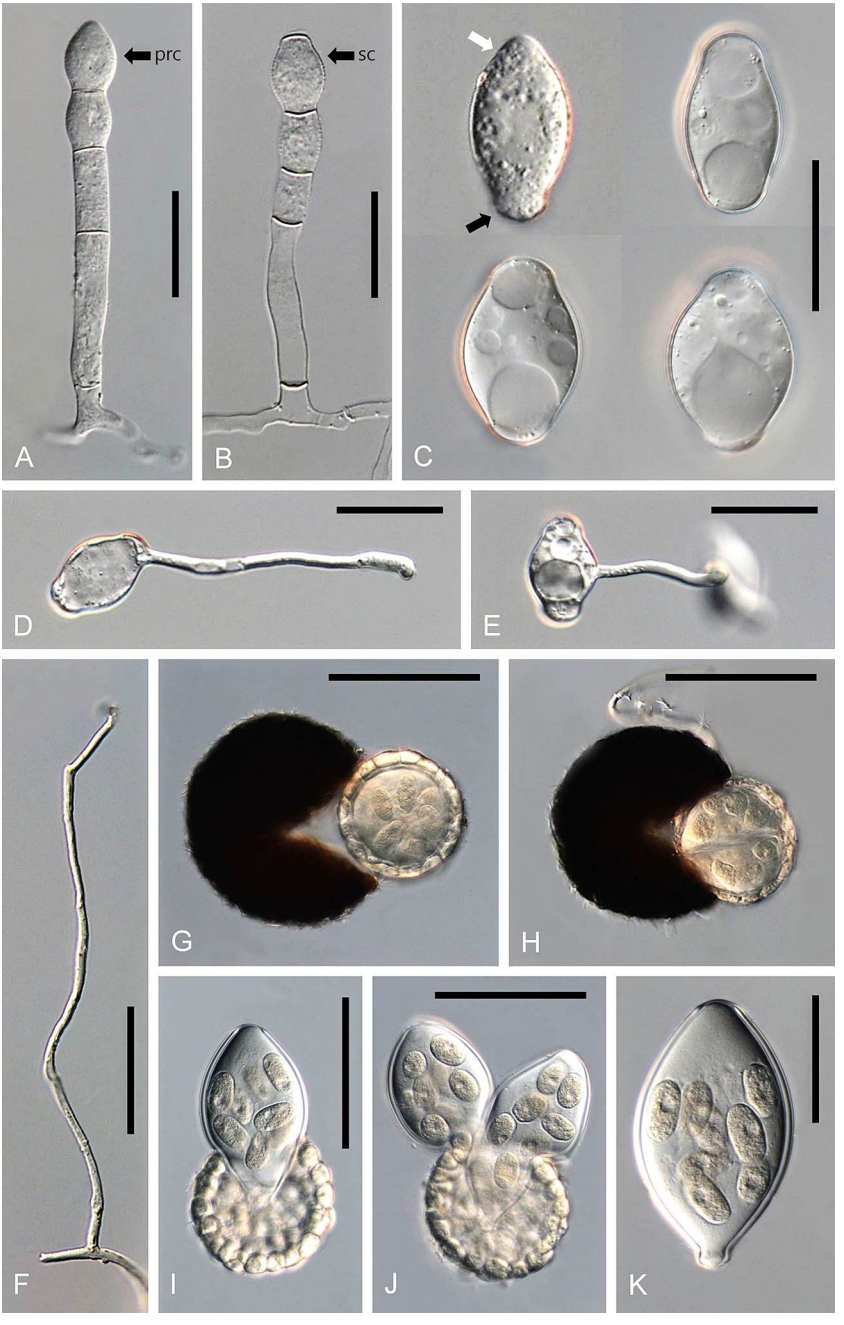
Fig. 1. *Cystotheca kusanoi*. A: Type collection (TNS-F-197792) of *Cystotheca kusanoi* in National Museum of Nature and Science, Tsukuba, Japan; B: Chasmothecium containing single ascus; C, D: Single ascus in an inner walled chasmothecium; E: Ascus containing 8 ascospores. Scale bars: B = 100 µm; C–E = 50 µm.


TYPUS
NATIONAL SCIENCE MUSEUM, TOKYO
HERBARIUM OF CRYPTOGAMS
Sphaerotheca kusanoi P. Hennings et Shirai
on Quercus glandulifera Bl.
Miyougi
Coll. S. Kusano
No. F-197792
Date 4-XI-1899
Det.
A
B
C
D
E

hyaline, polygonal cells, 12.5–17.5 µm wide. Appendages mycelioid, few, interwoven with the mycelium, pale brown to rusty brown, 4–6.5 µm wide. Asci short-stalked, 80–110 × 57–65 µm, with a terminal oculus 12–15 µm wide, containing 8 ascospores. Ascospores hyaline, oblong-elliptical, 23–30 × 16–19 µm.

Specimens examined: **KOREA**, **Gangwon**, **Gangneung**, on *Q. variabilis*, 16 Jun. 1992, H.D. Shin (KUS-F11691; GenBank MG865450, MG865600); 22 Jun 1992, H.D. Shin (KUS-F11727; GenBank MG865451); 22 Jun 1992, H.D. Shin (KUS-F11729); 2 Aug 1992, H.D. Shin (KUS-F11827; GenBank MG865601); on *Quercus* sp., 1998, S. Takamatsu (VPRI 20287, GenBank AF073354); **Goseong**, on *Q. serrata*, 3 Sep 2012, H.D. Shin & S.E. Cho (KUS-F26873; GenBank MG865455, MG865606); on *Q. variabilis*, 3 Sep 2012, H.D. Shin & S.E. Cho (KUS-F26874; GenBank MG865456, MG865607); **Samcheok**, on *Q. variabilis*, 11 Oct 2009, H.D. Shin (KUS-F24728; GenBank MG865453, MG865603); 4 Sep 2012, H.D. Shin & S.E. Cho (KUS-F26885; GenBank MG865457, MG865608); 28 Aug 2011, H.D. Shin & S.E. Cho (KUS-F26041; GenBank MG865605); on *Q. serrata*, 15 Jun 2015, H.D. Shin & S.E. Cho (KUS-F28637; GenBank MG865459); on *Q. variabilis*, 15 Jun 2015, H.D. Shin & S.E. Cho (KUS-F28638; GenBank MG865460); 7 Sep 2015, H.D. Shin & S.E. Cho (KUS-F28817); on *Q. serrata*, 7 Sep 2015, H.D. Shin & S.E. Cho (KUS-F28818); on *Q. variabilis*, 7 Sep 2015, H.D. Shin & S.E. Cho (KUS-F28826; GenBank MG865461, MG865610); 20 Oct 2015, H.D. Shin & S.E. Cho (KUS-F28987; GenBank MG865462, MG865611); on *Q. serrata*, 20 Oct 2015, H.D. Shin & S.E. Cho (KUS-F28988, GenBank MG865463, MG865612); on *Q. variabilis*, 20 Oct 2015, H.D. Shin & S.E. Cho (KUS-F28989); **Sokcho**, on *Q. variabilis*, 13 Sep 2009, H.D. Shin (KUS-F24556); **Yangyang**, on *Q. serrata*, 26 Jul 2013, H.D. Shin & S.E. Cho (KUS-F27389; GenBank MG865458, MG865609); **South Gyeongsang**, **Namhae**, on *Q. serrata*, 30 Jun 2004, H.D. Shin (KUS-F20369; GenBank MG865452, MG865602); **North Jeolla**, **Gimje**, on *Q. serrata*, 13 Jul 2010, H.D. Shin (KUS-F25062; GenBank MG865454, MG865604); **JAPAN**, **Fukushima**, 31 Oct 1953, Y. Nomura (TNS-F-128983); **Gunma**, on *Q. glandulifera* [= *Q. serrata*], 4 Nov. 1899, S. Kusano no. 123 (TNS-F-197792, isotype); 22 Nov 1980, S. Tozuka (TUAT 1675); on *Q. mongolica*, 22 Nov 1980, S. Tozuka (TUAT 1739); **Ishikawa**, on *Q. mongolica*, 1 Aug 1973, S. Kakimoto (TUAT 131); on *Q. serrata*, 27 Sep 1980, S. Tanda & Yamazaki (TUAT 1286; GenBank MG865465, MG865614); 27 Sep 1980, Y. Nomura (TNS-F-129021, 129029); **Kanagawa**, on *Q. serrata*, 6 Nov 1977, Y. Nomura (TNS-F-128986); **Nagano**, on *Q. serrata*, 1 Oct 1987, S. Tanda & al. (TUAT 3882); on *Q. mongolica*, 22 Sep 1983, Y. Nomura (TNS-F-128991); on *Q. serrata*, 24 Sep 1983, S. Tanda & al. (TUAT 2196); 24 Sep 1983, Y. Nomura (TNS-F-129027); **Niigata**,

Fig. 2. *Cystotheca kusanoi*. A: Conidiophore: note the conically rounded apex of the uppermost immature primary conidium (prc); B: Conidiophore; note the subtruncate apex of the uppermost immature secondary conidium (sc); C: Conidia; a primary conidium is displayed in the upper left. Note the conically rounded apex (white arrow) and subtruncate base (black arrow), giving horizontal asymmetry; Other three conidia are secondary conidia with horizontal symmetry; D, E: Germinating conidia; F: Special aerial hypha; G: Chasmothecium containing single ascus; H: Chasmothecium containing two asci; I: Single ascus in an inner walled chasmothecium; J: Two asci in an inner walled chasmothecium; K: Ascus containing 8 ascospores. Scale bars: A–E = 30 µm; F, K = 50 µm; G–J = 100 µm.


prc
sc
A
B
C
D
E
F
G
H
I
J
K

on *Q. serrata*, 2 Nov 1979, S. Tanda (TUAT 1200); **Sendai**, on *Q. mongolica*, 28 Sep 1973, Y. Nomura (TNS-F-128981); **Shizuoka**, on *Q. serrata*, 1 Oct 1973, Y. Nomura (TNS-F-128996); **Tokyo**, on *Q. serrata*, 20 Jul 1976, Y. Nomura (TNS-F-128994); 5 Sep 1976, Y. Nomura (TNS-F-128987, 129006); 19 Oct. 1980, S. Tanda & Yamazaki (TUAT 1351); **Yamagata**, on *Q. serrata*, 21 Oct 1977, S. Sato (TUAT 511; GenBank MG865464, MG865613); on *Q. mongolica*, 21 Oct 1977, S. Sato (TUAT 539); 28 Aug 1987, Y. Nomura (TNS-F-128982); **Yamanashi**, on *Q. serrata*, 27 Oct 1957, Y. Nomura (TNS-F-128989, 129007); 3 Oct 1976, Y. Nomura (TNS-F-128990, 129025); 23 Oct 1977, Y. Nomura (TNS-F-128988); 6 Nov 1977, Y. Nomura (TNS-F-128997); 23 Oct 1979, Y. Nomura (TNS-F-129001); 1 Aug 1980, Y. Nomura (TNS-F-128993); 28 Oct 1980 Y. Nomura (TNS-F-128985); n.d., Y. Nomura (TNS-F-128928).

White mycelial mats were observed on the abaxial surface of the leaves, and the persistent mycelium later formed a dense brownish felt on the leaves.

Cystotheca lanestris is morphologically similar to *C. kusanoi* but differs by its longer (130–280 µm) conidiophores with longer (30–110 µm) foot-cells. The presence or absence of fibrosin bodies has not been described in detail in *Cystotheca,* and fibrosin bodies were not found in the fresh Korean specimens of *C. kusanoi.* Therefore, conidia and conidiophores should be examined in fresh *Cystotheca* specimens to confirm the existence of fibrosin bodies in the genus. The fibrosin lineage, including *Cystotheca*, *Podosphaera*, and *Sphaerotheca*, contains only a single ascus per chasmothecium (Braun 1987, Braun & Cook 2012). While Korean collections contained rarely two asci per chasmothecium, chasmothecia in Japanese collections consistently contained a single ascus.

Phylogeny

A BLASTn search with sequences retrieved from the Korean *Cystotheca* specimens against the NCBI nucleotide database showed that the Korean taxon is most closely related to *C. lanestris* (GenBank AB000933, AF011288, AF011289, MF170000). The phylogenetic trees constructed with the ITS and 28S rDNA region dataset supported the Korean and Japanese *Cystotheca* collections in an independent lineage that differed from *C. lanestris* collected in North America. In addition, the Asian species was supported as sister to *C. lanestris*, implying a close phylogenetic relationship between the two species, thereby confirming the phylogenetic position of *C. kusanoi* as a separate species in this study (Figs 3, 4). Although *C. kusanoi* is phylogenetically close to *C. lanestris*, DNA sequence comparisons based on the ITS and 28S rDNA gene regions clearly distinguish the two species, supported by strong bootstrap values, and endorse the hypothesis that they diverged from a common ancestor. GenBank AF073354 was recorded as *C. lanestris* on *Quercus* sp. from Korea (Cunnington & al. 2003), but

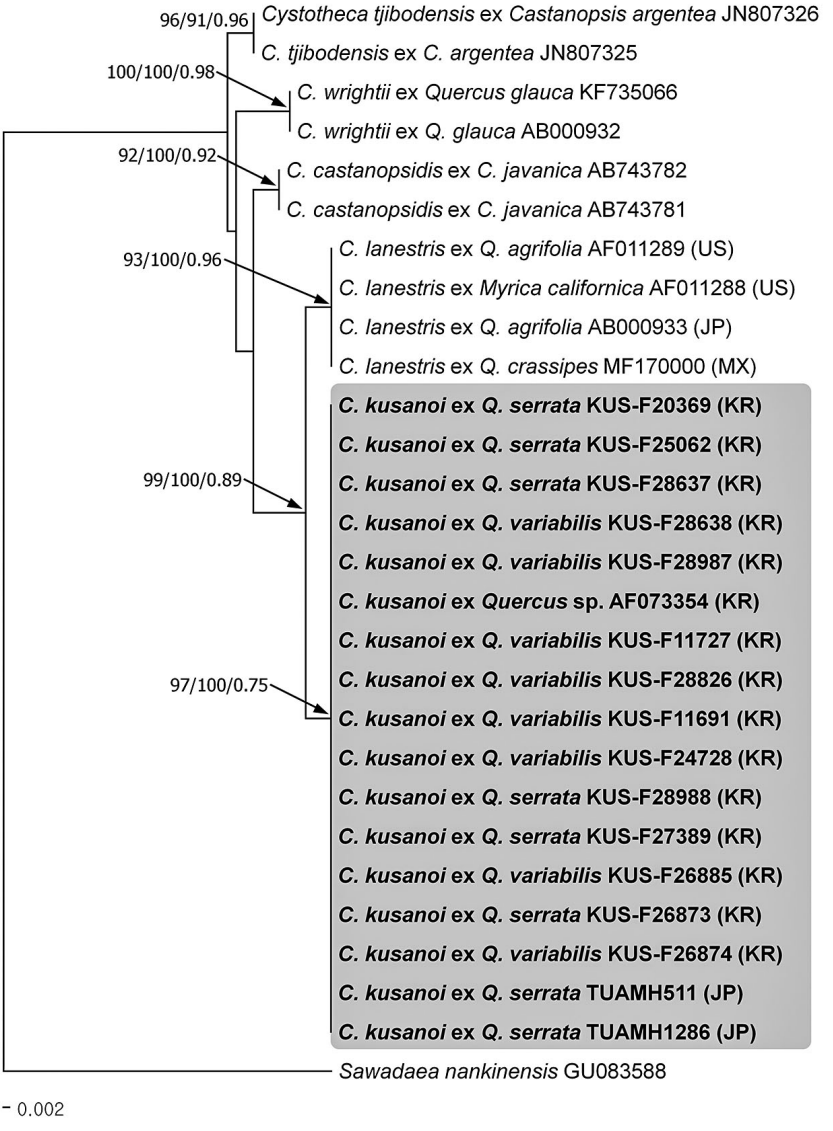

96/91/0.96
Cystotheca tjibodensis ex Castanopsis argentea JN807326
C. tjibodensis ex C. argentea JN807325
100/100/0.98
C. wrightii ex Quercus glauca KF735066
C. wrightii ex Q. glauca AB000932
92/100/0.92
C. castanopsidis ex C. javanica AB743782
C. castanopsidis ex C. javanica AB743781
93/100/0.96
C. lanestris ex Q. agrifolia AF011289 (US)
C. lanestris ex Myrica californica AF011288 (US)
C. lanestris ex Q. agrifolia AB000933 (JP)
C. lanestris ex Q. crassipes MF170000 (MX)
C. kusanoi ex Q. serrata KUS-F20369 (KR)
C. kusanoi ex Q. serrata KUS-F25062 (KR)
99/100/0.89
C. kusanoi ex Q. serrata KUS-F28637 (KR)
C. kusanoi ex Q. variabilis KUS-F28638 (KR)
C. kusanoi ex Q. variabilis KUS-F28987 (KR)
C. kusanoi ex Quercus sp. AF073354 (KR)
C. kusanoi ex Q. variabilis KUS-F11727 (KR)
97/100/0.75
C. kusanoi ex Q. variabilis KUS-F28826 (KR)
C. kusanoi ex Q. variabilis KUS-F11691 (KR)
C. kusanoi ex Q. variabilis KUS-F24728 (KR)
C. kusanoi ex Q. serrata KUS-F28988 (KR)
C. kusanoi ex Q. serrata KUS-F27389 (KR)
C. kusanoi ex Q. variabilis KUS-F26885 (KR)
C. kusanoi ex Q. serrata KUS-F26873 (KR)
C. kusanoi ex Q. variabilis KUS-F26874 (KR)
C. kusanoi ex Q. serrata TUAMH511 (JP)
C. kusanoi ex Q. serrata TUAMH1286 (JP)
Sawadaea nankinensis GU083588
0.002

Fig. 3. Phylogenetic tree for *Cystotheca* inferred from maximum likelihood analysis based on ITS regions. MPBS, MLBS, and Bayesian PP above 50% are given above or below the branches. The number of nucleotide changes among taxa is represented by branch length, and the scale bar represents the number of nucleotide substitution per site.

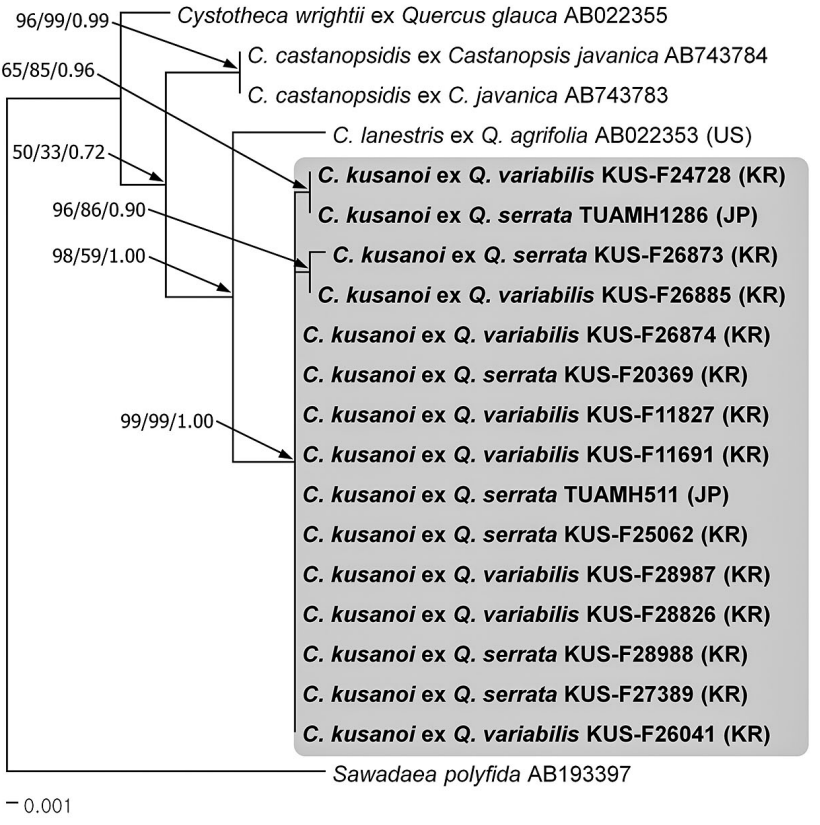

96/99/0.99
Cystotheca wrightii ex Quercus glauca AB022355
C. castanopsidis ex Castanopsis javanica AB743784
65/85/0.96
C. castanopsidis ex C. javanica AB743783
C. lanestris ex Q. agrifolia AB022353 (US)
50/33/0.72
C. kusanoi ex Q. variabilis KUS-F24728 (KR)
96/86/0.90
C. kusanoi ex Q. serrata TUAMH1286 (JP)
98/59/1.00
C. kusanoi ex Q. serrata KUS-F26873 (KR)
C. kusanoi ex Q. variabilis KUS-F26885 (KR)
C. kusanoi ex Q. variabilis KUS-F26874 (KR)
C. kusanoi ex Q. serrata KUS-F20369 (KR)
99/99/1.00
C. kusanoi ex Q. variabilis KUS-F11827 (KR)
C. kusanoi ex Q. variabilis KUS-F11691 (KR)
C. kusanoi ex Q. serrata TUAMH511 (JP)
C. kusanoi ex Q. serrata KUS-F25062 (KR)
C. kusanoi ex Q. variabilis KUS-F28987 (KR)
C. kusanoi ex Q. variabilis KUS-F28826 (KR)
C. kusanoi ex Q. serrata KUS-F28988 (KR)
C. kusanoi ex Q. serrata KUS-F27389 (KR)
C. kusanoi ex Q. variabilis KUS-F26041 (KR)
Sawadaea polyfida AB193397
0.001

Fig. 4. Phylogenetic tree for *Cystotheca* inferred from maximum likelihood analysis based on the 28S region. MPBS, MLBS, and Bayesian PP above 30% are given above or below the branches. The number of nucleotide changes among taxa is represented by branch length, and the scale bar represents the number of nucleotide substitution per site.

our analyses place this specimen in the *C. kusanoi* clade while GenBank AB000933, derived from material collected at the University of California-Berkeley campus in 1995 by S. Takamatsu, is in the *C. lanestris* clade.

Discussion

Harkness (1884) described *Sphaerotheca lanestris* based on a Californian specimen found on *Quercus agrifolia* Née, an evergreen oak, and Salmon (1900) discussed and described in detail the species in his monograph of *Erysiphaceae*.

Hennings (1900) proposed the new species *S. kusanoi* for a specimen found on *Q. glandulifera* [= *Q. serrata*] collected in Japan by Kusano. Later, *Sphaerotheca lanestris* was transferred to the genus *Cystotheca* under the name *C. lanestris* and *S. kusanoi* was regarded as a synonym of *C. lanestris* (Ideta 1909).

Harkness (1884) described the anamorph of *Cystotheca lanestris* as:

> "*Oidium ventricosum* Harkn.; segment swelling in the center and becoming barrel-shaped, 34–38 × 20–22 µm, and filled with numerous round or elliptic bodies, 5–6 × 2–4 µm, which are freely discharged from the end, as the joints separate."

Sawada (1915) and Homma (1937) made similar observations. Meeboon & al. (2012) showed that *C. tjibodensis* has rather inconspicuous fibrosin bodies; and characterized *Cystotheca castanopsidis* as having rather inconspicuous fibrosin bodies (Meeboon & al. 2013, Wijayawardene & al. 2017). The description by Shin (1991, 2000) confirmed that powdery mildew species from Korea do not contain distinct fibrosin bodies in the conidial state. Furthermore, the descriptions and photographs of *Cystotheca wrightii* on *Quercus glauca* Thunb., recently reported from Korea by La & al. (2014), confirm that fibrosin bodies are also absent in the fresh conidia and conidiophores of that species. Thus, it appears that some *Cystotheca* species of do not contain any conspicuous fibrosin bodies in their fresh conidia and conidiophores, and additional studies are needed to confirm these peculiarities in *Cystotheca*.

According to descriptions made by Shin (1991), the Korean *Cystotheca* specimens are similar to *C. lanestris* with regard to the symptoms produced and host species. However, Shin's (1991) examinations and observations in our study suggest that Korean *Cystotheca* specimens on *Quercus* spp. represent a separate species, clearly different from *C. lanestris* and which can be referred to as *C. kusanoi*, based on the previously described *Sphaerotheca kusanoi*. During extensive field forays searching for powdery mildew fungi, particularly for *Cystotheca* spp., *Q. serrata*, and *Q. variabilis* Blume were identified as Korean hosts of *C. kusanoi*. Since *Q. variabilis* has often been misidentified as "*Q. acutissima* Carruth." in Korea, we cannot infer how wide the host range of the fungus might be in nature. In Japan, *Q. serrata* and *Q. mongolica* Fisch. ex Ledeb. were recorded as host species of *C. kusanoi*. The identity of other host plants remains unclear, and some collections on hosts recorded in the literature were unavailable for re-examination in Korea.

Our present morphological re-examinations and molecular analyses clearly support the new combination *C. kusanoi* for the powdery mildew fungus infecting *Quercus* spp. in Korea and Japan.

Key to *Cystotheca* species all known on *Fagaceae*

(except as otherwise annotated)

1. Mycelium amphigenous 2
1. Mycelium hypophyllous 3
2. Hyphal appressoria well developed, nipple-shaped ..... *C. castanopsidis*
2. Hyphal appressoria lobed ..... *C. quercina*
3. Special aerial hyphae absent ..... *C. esetacea*
3. Special aerial hyphae present 4
4. Special aerial hyphae curved to falcate ..... *C. wrightii*
4. Special aerial hyphae lacking or filiform 5
5. Chasmothecial appendages numerous, well developed, long; non-fagaceous host ..... *C. indica*
5. Chasmothecial appendages lacking or few, poorly developed, short 6
6. Mycelial patches dark brown or purplish brown 7
6. Mycelial patches whitish to light brown 8
7. Chasmothecia 90–130 µm diam. ..... *C. tjibodensis*
7. Chasmothecia smaller, 60–70 µm diam. ..... *C. nanyuensis*
8. Fresh conidia without distinct fibrosin bodies ..... *C. kusanoi*
8. Fresh conidia with distinct fibrosin bodies ..... *C. lanestris*

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